Results: No animals showed any neurologic abnormalities before re-irradiation. RM occurred in 22 control animals after a median latency of 117 days (92-212 days) from second dose. In contrast, only 5 treated rats developed RM (after 108-174 days) within 270 days, $p<0.05$. ED50 was 18.5 Gy (95% confidence interval 17.2-19.6 Gy) in the control group versus 24.6 Gy (22.1-58 Gy) in the treatment group. However, within comparably irradiated groups, i.e. 17-23 Gy, 11 rats receiving IGF-1 plus amifostine (6/11 received 23 Gy) versus none of the control rats died of unknown causes within 30 days after re-irradiation. Gross and histopathologic lesions in these rats that died unexpectedly were insufficient to determine the cause of death.

Conclusion: The experimental data revealed supra-additive effects of IGF-1 and amifostine in reducing radiation neurotoxicity resulting in increasing the ED50 by more than 30%. This finding strengthens the evidence that brief therapeutic intervention can decrease radiation-induced neurotoxicity. However, unexpected from our earlier study in previous unirradiated rats, the regimen also induced mortality in re-irradiation setting. Further studies will be undertaken to optimize the regimen.

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EGF-receptor tyrosine kinase inhibition combined with fractionated radiotherapy in human squamous cell carcinoma xenografts

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Purpose: Proliferation of clonogenic tumour cells during fractionated irradiation is a major cause of local failure in squamous cell carcinoma (SCC). The EGFR signal transduction pathway has been suggested to play an important regulative role in this process. The aim of our study was to investigate whether specific inhibition of the EGFR-TK by BIBX1382BS improves the results of fractionated irradiation of EGFR-positive FaDu hSCC in nude mice.

Methods: Proliferation rate, cell cycle distribution and BrdUrd-LI, and clonogenic cell survival were determined in vitro after application of 5 µmol/l BIBX1382BS or carrier. Tumor-bearing nude mice received BIBX1382BS (50 mg/kg/d) alone or simultaneously with fractionated RT. Experimental endpoint was tumor growth delay. In addition histological investigations on BrdU-LI, Ki67-LI, necrosis and apoptosis were performed.

Results: In line with histological and FCM results showing a decreased BrdUrd labelling and accumulation of cells in G1, BIBX1382BS significantly decreased the growth of FaDu cells in vitro and of FaDu tumors in nude mice. In vitro BIBX1382BS was slightly cytotoxic. When given simultaneously to 15x2 Gy, BIBX1382BS had no effect on tumor growth delay.

Conclusion: EGFR-TK inhibitor BIBX1382BS significantly decreases proliferation of FaDu tumors. Results after a short course of fractionated RT were not improved. However, as repopulation of clonogenic cells in FaDu tumors has been shown to accelerate after 3 weeks of fractionated RT, it appears possibly that combined treatment may be more effective after longer overall treatment times. This question is currently investigated.

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568 ORAL

Reduced DNA-dependent protein kinase activity in two cell lines derived from individuals with radionecrosis

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Background: Late normal tissue toxicity limits the dose of radical radiotherapy. In mammalian models, radiosensitivity is almost invariably associated with DNA repair defects. To investigate the role of this phenotype in late radionecrosis, we have examined the activity of enzymes involved in non-homologous endjoining (NHEJ) and double-strand break repair in cell lines derived from patients with late radiation injury.

Aim: To assess the effect of NHEJ enzyme activity on late radiation injury.

Methods: Patients with necrosis (grade 4 or 5 RTOG late morbidity) after radical radiotherapy were identified from the departmental database of patients treated since 1974. Sections from paraffin-fixed archival blocks were stained with antibodies against enzymes involved in NHEJ. EBV-transformed lymphoblastoid cell lines were derived from 5 patients who sustained injury at "safe" doses. Control cell lines were obtained from 3 patients without cancer. Post-radiation viability was assessed by colorimetric absorbance. DNA-dependent protein kinase (DNA-PK) activity was assayed with biotinylated peptide substrate. NHEJ enzyme expression was determined by immunoblotting.

Results: Post-radiation viability in cell lines (LB0003 and LB0004) derived fron two patients with radionecrosis was intermediate between an ataxia-telangiectasia cell line and normal controls. These two cell lines exhibited 8-fold reduction in DNA-PK activity. Sections from a post-radiation cervix biopsy in one patient, and the bilateral breast cancers in the second, showed no evidence of staining with antibodies against DNA-PKcs. NHEJ enzymes were expressed in all cell lines.

Conclusion: These data suggest reduced DNA-PK activity may be implicated in late radiation injury in some patients.

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Combination of the TRAIL death ligand with ionizing radiation - rationale and efficacy

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Rationale: A combination of antitumor approaches acting on different death pathways seems ideal for increasing therapeutic responses, especially when defined resistance mechanisms interfere with individual cellular processes.

Materials and methods: Apoptosis induced by TRAIL or ionzing radiation (XRT) alone or in combination was analyzed by FACS. Caspase-8/-9 and BID activation was analyzed by western blotting. Mitochondiral damage was inhibited by overexpression of Bcl-2

Results: Both TRAIL and XRT induced activation of caspase-8, caspase-3, BID and mitochondrial potential loss. TRAIL induced apoptosis required caspase-8, whereas it was not essential for radiation induced apoptosis. Inhibition of mitochondrial damage by Bcl-2 abrogated XRT induced apoptosis and caspase activation, but attenuated TRAIL induced apoptosis only. Combined treatment TRAIL/XRT exerted additive apoptotic effects in control cells, whereas synergistic effects occurred in cells overexpressing Bcl-2. A strong effect of TRAIL on radiation induced clonogenic cell death was found. Similar data were obtained with solid tumor lines (MCF-7, R30C, Colo 824, BT474 (Breast) A549 (Lung) FaDu, SCC4, SCC9 (H&N) HT29 (Rectum). All lines except Colo 824 were apoptosis resistant when irratiated with 10 Gy. However TRAIL induced cell death in SCC4, R30C, HT 29, A549, BT474, FaDu. No response was detectable in fibroblasts. Preirradiation induced strongly increased TRAIL effects in R30C and SCC4 cells

Conclusion: The TRAIL death ligand seems to be of high potential value for a combination with ionizing radiation in tumor therapy.

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Evidence for the p53 tumour suppressor protein as a direct sensor of DNA damage

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Mammalian cells delay their cell cycle progression after DNA damage (ie. G1 and G2 cell-cycle checkpoints), presumably to allow time for DNA repair, thereby maintaining their genomic integrity. Molecular data exists to suggest that focal DNA repair protein-protein interactions (ie. rad50-mre11; rad51-BRCA1) occur within the nuclei of irradiated cells at sites of DNA-dsb following IR, but whether these focal interactions occur secondary to direct signals and interactions with DNA damage checkpoint sensing protein (ie. p53, ATM) is unknown. Indeed, the wild type p53 G1-checkpoint can be activated with as little as one DNA-dsb and cause a permanent G1 arrest in lethally irradiated fibroblasts. As yet, direct evidence that the p53 protein can sense and activate DNA-dsb repair following irradiation as part of a DNA damage checkpoint response is lacking. To test the hypothesis that the p53 protein can sense DNA breaks in vivo, we have obtained data using quantitative immunofluorescence, confocal microscopy with antibodies to specific phospho-forms of p53. In a dose-responsive manner, normal human fibroblasts irradiated in plateau phase (ie. GM05757) show an accumulation of discrete nuclear foci when stained with an antibody to the serine-15 phosphorylated form of p53 (ie. ser15-p53) which is a form activated by IR in an ATM-dependent manner. Dose-responsive foci can be observed within 30 minutes of IR-exposure, suggesting that p53 rapidly localizes to sites of IR-induced damage. A kinetic study of ser15-p53 accumulation in GM05757 cells suggest that despite a rapid induction of ser15-p53 following IR, a high level of residual foci remain at 24 hours which correlates to the level of rad50 foci. Rad51 foci are not dose-responsive and are